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Direct Colorimetric Detection of a Receptor-Ligand Interaction by a Polymerized Bilayer Assembly

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lipid uniformly disperses the sialoside lipid, which allows optimum binding of the virus (25). The mixed monolayer was compressed and polymerized on the water surface. The floating polymerized assembly was lifted by the horizontal touch method onto a glass slide previously coated with a self-assembled monolayer of OTS (26). The resulting bilayer assembly presents an array of carbohydrate ligands at the surface. The tetraethylene glycol spacer in sialoside lipid **2** serves to extend the carbohydrate ligand beyond the carboxylic acid head groups of the matrix lipid **1**.

Films prepared in this manner exhibit a high degree of order over a macroscopic range (50 to 150 μM) as evidenced by optical microscopy with the use of crossed polarizers (27) (Fig. 2B). The films were further characterized by angle-resolved x-ray photoelectron spectroscopy (XPS) and ellipsometry. The XPS results indicate that the amide nitrogen atoms and the carbonyl carbon atoms of the head groups are localized at the surface relative to the methylene carbons of the lipid chains, demonstrating that the sialoside head group is presented at the surface of the film. Ellipsometric analysis of the polydiacetylene monolayer coated on HF-treated silicon indicates a film thickness of ~40 Å, in agreement with the expected value based on molecular modeling.

The bilayer assembly has a visible absorption maximum of 620 nm and appears as a blue film. When the film is incubated with X31 influenza A virus [PBS (phosphate-buffered saline) buffer, pH 7.4], the binding of the viral hemagglutinin to the sialic acid residues on the surface results in a blue to red color transition (Fig. 3A). No color change is observed when the blue film is incubated with a blank solution of PBS buffer. This result demonstrates a polydiacetylene color transition arising from affinity binding (affinitychromism) rather than thermal annealing (thermochromism). Previous studies have shown that LB films composed of lipid 1 undergo a blue to red color change when heated at 70°C, which corresponds to the endothermal transition for lipid chain melting (28). Lipid chain disorder and tangling decrease the effective conjugation length of the polydiacetylene backbone. Similarly, Fourier transform infrared (28, 29) and resonance Raman spectroscopy (29) as well as x-ray data (30, 31) demonstrate that lipid chain packing of the red form of the polymer is different from that of the blue form. Thus, conformational changes in the lipid chains affect the optical properties of the polymer backbone. Binding of the viral hemagglutinin to the sialoside bilayer assembly appears to affect the lipid chain conformations in a manner analogous to thermal annealing.

In addition to qualitative evaluation by visual inspection, the degree of color change is readily quantified by standard visible absorption spectroscopy (Fig. 3B). The blue-colored film has a strong absorption maximum at 620 nm and a weaker absorption at 550 nm. After incubation with influenza virus, a dramatic change in the visible absorption spectrum occurs. The maximum at 550 nm increases with a con-

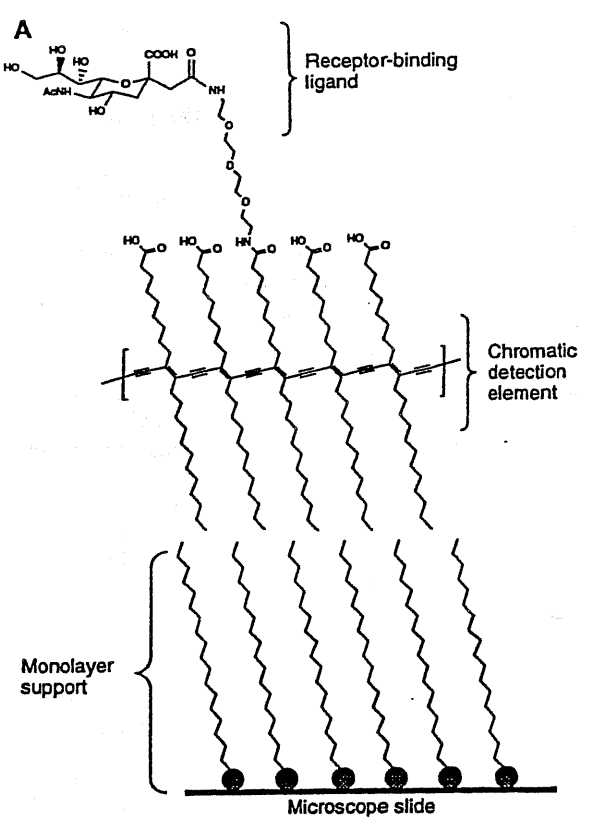

Fig. 2. Film structure and morphology. **(A)** Schematic diagram of the polymerized bilayer assembly. The siloxane linkages of the bottom monolayer are not shown. **(B)** Optical micrograph of the sialoside bilayer assembly between crossed polarizers. Large domains up to 150 μM are visible. Scale: 1 cm = 20 μM.

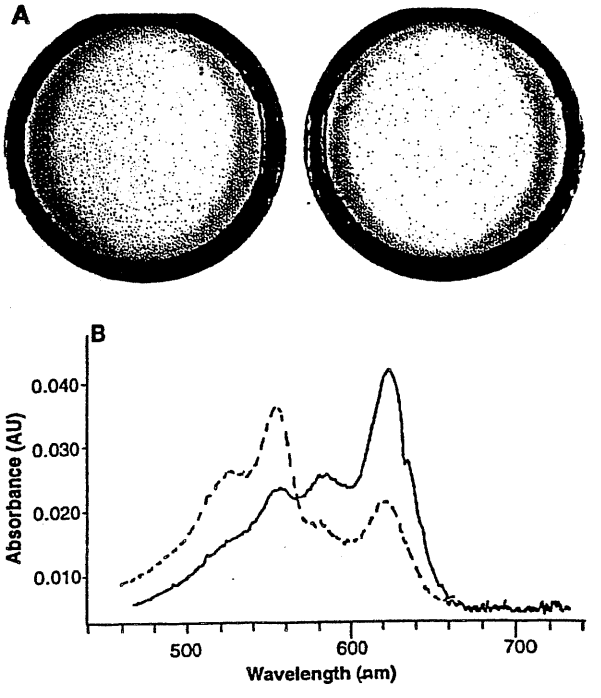

Fig. 3. Colorimetric detection of influenza by sialoside bilayer assembly (2% sialoside lipid **2** and 98% matrix lipid **1**). **(A)** The colorimetric response of the film, supported on a glass microscope slide, is readily visible to the naked eye for qualitative evaluation of the presence of virus. The film on the left (blue) has been exposed to a blank solution of PBS. The film on the right (red) has been exposed to 100 HAUs of virus (CR = 77%, see text). A colorimetric response of ~15% can be observed visually. **(B)** The visible absorption spectrum of a bilayer assembly prior to (solid line) and after (dashed line) viral incubation. The bilayer assembly was inserted into a quartz cuvette containing PBS buffer (pH 7.4), and the absorption spectrum was obtained. Addition of influenza virus in PBS buffer (pH 7.4) resulted in a chromatic transition following a 30-min incubation period. (Although the film color begins to change within seconds after exposure to virus, 30 min was found to be the average length of time required for the CR to reach a plateau value in a nonstirred solution). These spectra represent a CR of 50%.

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25. We have previously shown that 1 to 5% of sialoside lipid gives maximum binding of the virus to polymerized liposomes (*24*). Ideal mixing of the two components was determined by analysis of the Langmuir isotherms. Various ratios of monomers **1** and **2** give isotherms whose limiting areas and collapse pressures change in direct proportion to the mole fraction of **2** as expected for miscibility [see (*3*)].
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36. The above experiments define a background level of CR arising from nonspecific adhesion. Thus, from Fig. 4A, the minimum quantity of virus that is detectable and well above this background level is ~25 HAUs. According to some estimates (*35*), this value corresponds to ~200 million particles. Given a cross-sectional area of $\sim 8 \times 10^{-11}$ cm^2 per particle, the minimum film area affected is ~0.02 cm^2, or 1/100 of the sample area. Given the high film response, it is likely that each binding event results in a much longer range induced disorder.
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